Serial No. 09/970,477 Amdt. dated June 13, 2003 Reply to Office Action of February 13, 2003

Amendments to the Specification:

Please amend the paragraph beginning at page 1, line 1, with the following paragraph:

-- This is a continuation of co-pending application Serial No. 09/210,168, now U.S. Patent No. 6,355,424, filed December 11, 1998 which claims benefit of Serial No. 60/082,167, filed April 17, 1998; Serial No. 60/070,486, filed January 5, 1998; and Serial No. 60/069,426, filed December 12, 1997. --

Please amend the paragraph beginning at page 14, line 17, with the following paragraph:

In one embodiment of the present invention RNA was analyzed directly by solution based procedures. The cells were first lysed by adding a proteolytic enzyme to the cells contained in wells of a microtiter plate. Non-limiting examples of enzymes for use in the present invention include proteinase K or Pronase. Cells can also be subjected to detergent lysis or osmotic lysis or a French Press. After incubation, biotinylated DNA probes were added to each well. The RNA:DNA hybrids were captured onto a solid phase by transferring to streptavidin coated microplates. Alkaline phosphatase-conjugated antibodies to RNA:DNA hybrids were added to each well in the hybridization microplate and signals were generated by adding a chemiluminescent reagent such as CDP-STAR® CDP-Star with Emerald II (Tropix) to each well. The signal was read from the microplate. The solution based DNA analysis was performed similarly to the RNA analysis except that the microtiter plates were coated with anti-RNA:DNA hybrid antibodies and the probes were RNA probes.

Please amend the paragraph beginning at page 18, line 9, with the following paragraph:

-- Cell samples for use in the present invention can be collected and stored in liquid medium. Examples of useful cell collection media are specimen transport medium (STM; (Digene), PRESERVCYT® PreservCyt (Cytyc), and CYTORICH® CytoRich (Autocyte). These media (PRESERVCYT® and CYTORICH® PreservCyt and CytoRich) were developed for the collection of cytological samples but can be adapted for use with molecular assays. --

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Please amend the paragraph beginning at page 21, line 15, with the following paragraph:

CaSki cell line was trypsinized by incubating with 0.25% Trypsin-EDTA for 5 minutes at 37 °C. Cells were pelleted from the suspension by centrifugation at 800 rpm for 3 minutes in Sorvall RT 6000 centrifuge. Cell pellet was resuspended in 500 μl of 1X PBS and counted under microscope using Trypan Blue solution. Cells were diluted to 50 and 500 cells/μ1 in 1X PBS. 10 μl of each cell concentration, including zero point (10 μl of 1X PBS) were spiked in 3 ml of PRESERVCYT® PreservCyt reagent. 100 μl of Sample Conversion Buffer were added into each tub to help visualize the cell pellet. All samples were mixed well and were centrifugedspun down at 3800 rpm for 15 minutes in Sorvall RT 6000 centrifuge. Supernatants were discarded and tubes were drained by inversion on the Kimtowels for 2 to 5 minutes on the bench. All pellets were resuspended with 50 μl of the lysis reagent (50 units of Proteinase K) and mixture was transferred into the plate coated with streptavidin. Plate was covered with the plate sealer and was incubated at 37°C for 30 minutes (heat block) with agitation every 15 minutes. --

Please amend the paragraph beginning at page 23, line 11 to page 24, line 2, with the following paragraph:

The RNA analysis was done according to Example 1 or the following procedure. Single stranded, biotinylated, DNA probes containing the specific HPV16 gene sequences were prepared. For HaCaT and SiHa cell lines, cells were grown to confluency, cells were harvested, and the total RNA was <u>isolated and purified using the RNEASY® RNeasy</u> kit (Qiagen Inc., Santa Clarita, CA). For W12, whole cells were used for analysis. RNA calibrators containing the complete HPV genome were prepared by transcribing (+) sense RNA from a plasmid containing the complete HPV16 genome with T7 RNA polymerase. The RNA was then diluted to 10³, 10⁴, 10⁵, 10⁶, and 10⁷ copies per 50 μl. Aliquots of cellular RNA were diluted to 50 μl and then 50 μl of Probe mix (containing the biotinylated, single-stranded DNA probe) was added to calibrators and cellular RNA and hybridized to the RNA specimens for 2 hours at 65 °C. The hybridization reactions were transferred to a

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streptavidin coated microplate and 25 µl of Detection Reagent 1 was added to each well. (Detection Reagent 1 contains the alkaline-phosphatase – anti-RNA:DNA monoclonal antibody conjugate.) During a 1 hour incubation with shaking, RNA:DNA hybrids were captured onto the streptavidin coated plate and were simultaneously reacted with the antihybrid antibody conjugate. After several wash steps, a chemiluminescent substrate (Tropix CDP-STAR® CDP star with Emerald) was added to the wells, and the light output was measured in a microplate luminometer after 30 minutes incubation at room temperature. - -